



# **NEAR REAL-TIME QUANTITATION OF VIABLE MICROORGANISMS FOR PLANETARY PROTECTION AND CREW HEALTH**

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# Needs for Rapid Biological Testing

- Quality Control
- Environmental Health
- Product release
- Microbiology
- Crew Health
- Infectious diseases
- Mission Science
- Astrobiology
- Planetary Protection
  - Spacecraft assembly / mission support

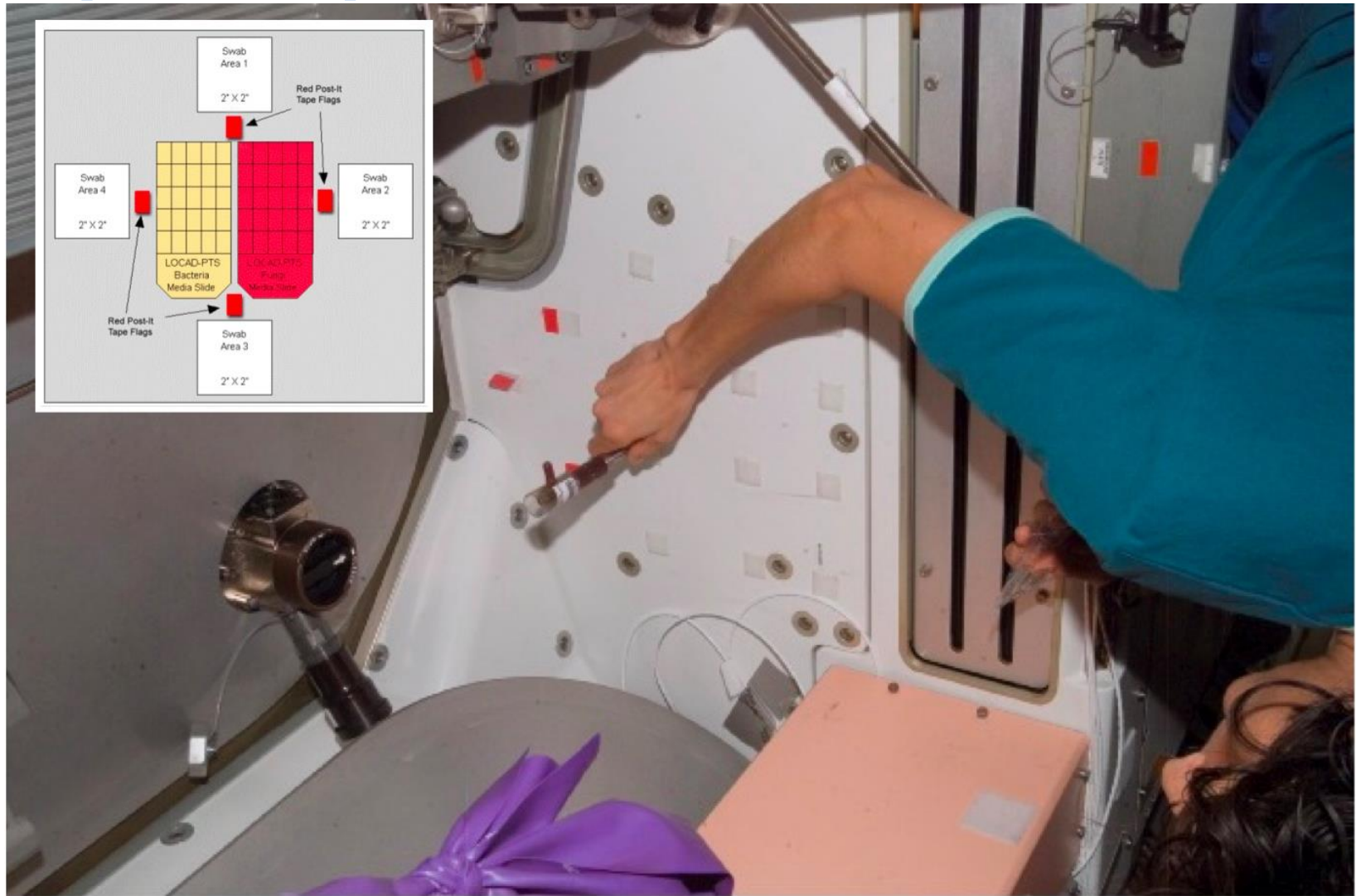


# Background

- Bacterial LPS / ATP as biomarker indicators of contamination
- Rapid Methods vs. Traditional Culture  
Minutes to results vs. Days to results
- LOCAD-PTS Lab on a Chip Application Development – Portable Test System



# Sample Acquisition on ISS

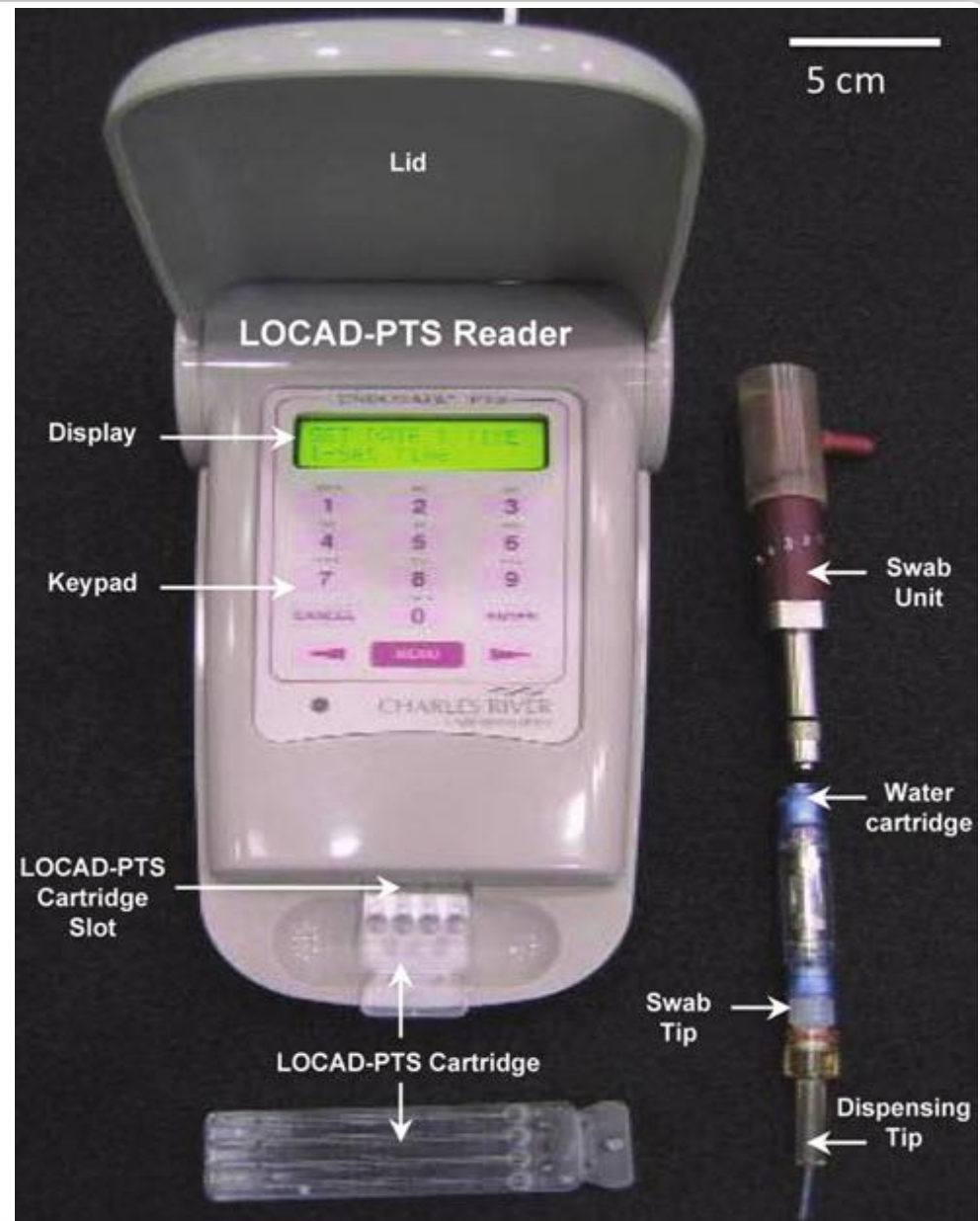


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# PTS™ Flight Unit



- **Pro: Rapid –**
  - Minutes vs days
  - No culture necessary
  - Smart – no user expertise needed
- **Con:**
  - No direct link to viable cells
  - No species information





# PTS Micro Scanner



# PTS Micro System

Small, rapid bioburden test system that directly measures viable microorganisms that have been isolated, stained and detected on a capillary or filter membrane.

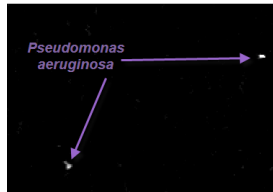


**Instrument w/ innovative  
high sensitivity optical  
signal detection**

## Flexible reagents based on application



# PTS Micro



## Live / dead cell differentiation staining

## Integrated, automated data analysis

Download CSV				
Amplitude			Position	
7	800 Hz	600 Hz		(cm)
7	780.0	786.7	7796.2	1523.9 5.1
8	8216.6	7349.1	8405.0	1675.0 0.4
9	8216.6	7349.1	8405.0	1675.0 0.4
10	8216.6	7349.1	8405.0	1675.0 0.4
11	8216.6	7349.1	8405.0	1675.0 0.4
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38	8216.6	7349.1	8405.0	1675.0 0.4
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60	8216.6	7349.1	8405.0	1675.0 0.4
61	8216.6	7349.1	8405.0	1675.0 0.4
62	8216.6	7349.1	8405.0	1675.0 0.4



# PTS Micro Reagents



- Filtration cup and funnel –
  - Adheres to current standard membrane filter procedure
  - 100ml volume, likely add 200 ml cup in future
  - Superior performance low background membranes
  - Gamma irradiated single sample consumable package
  - Optional enrichment step to further increase sensitivity

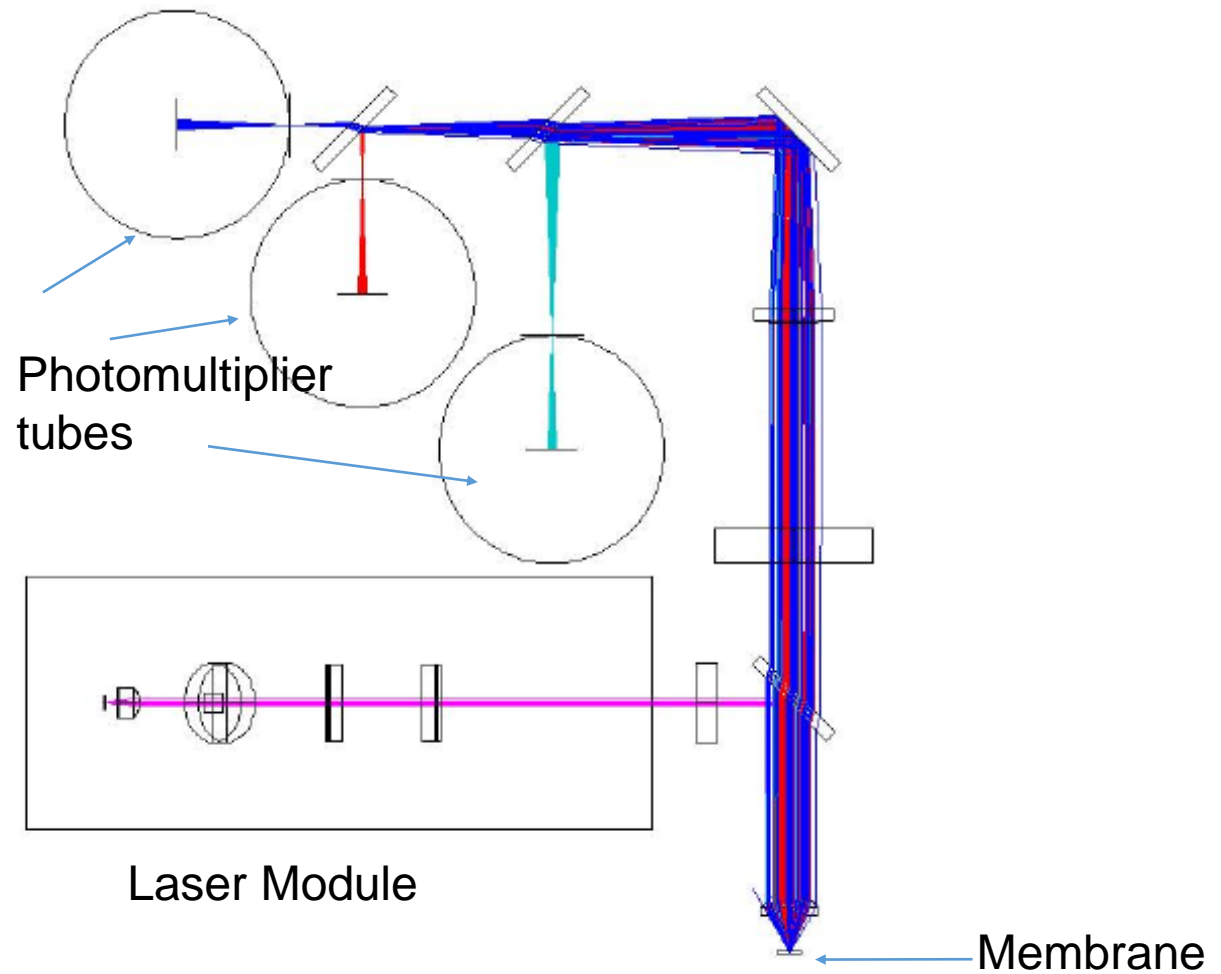
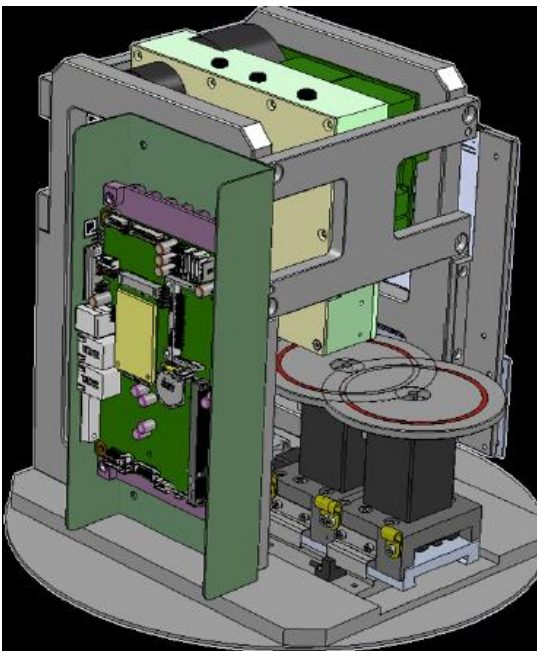
- Capillary
  - Direct measurement – no concentration
  - Bulk packaging – 50 / box
  - Sensitivity dependent on scan volume
  - Smaller scan area means faster scan time



- Positive control beads
  - Checks scanner precision, filter integrity
- Single use reagent dye
  - Live / dead cell differentiator

# Optics:

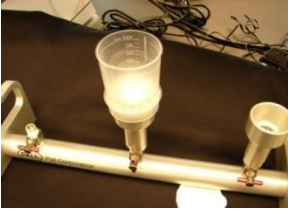
## Light path of Excitation / Emission



# PTS Micro Membrane Filter Procedure

## Laminar Flow Hood

### Step 1. Membrane Filtration



Step 2. Add Reagent Dye and incubate at room temp  
(optional enrichment step)

Step 3 Detach cup and cover membrane



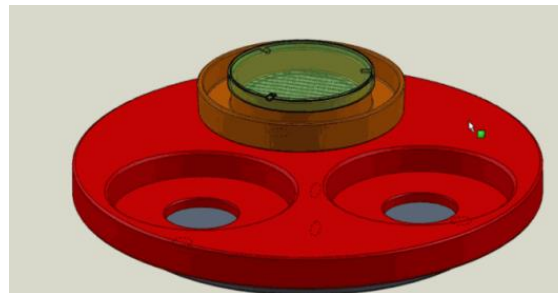
### Step 5. Scan



If positive,  
then culture

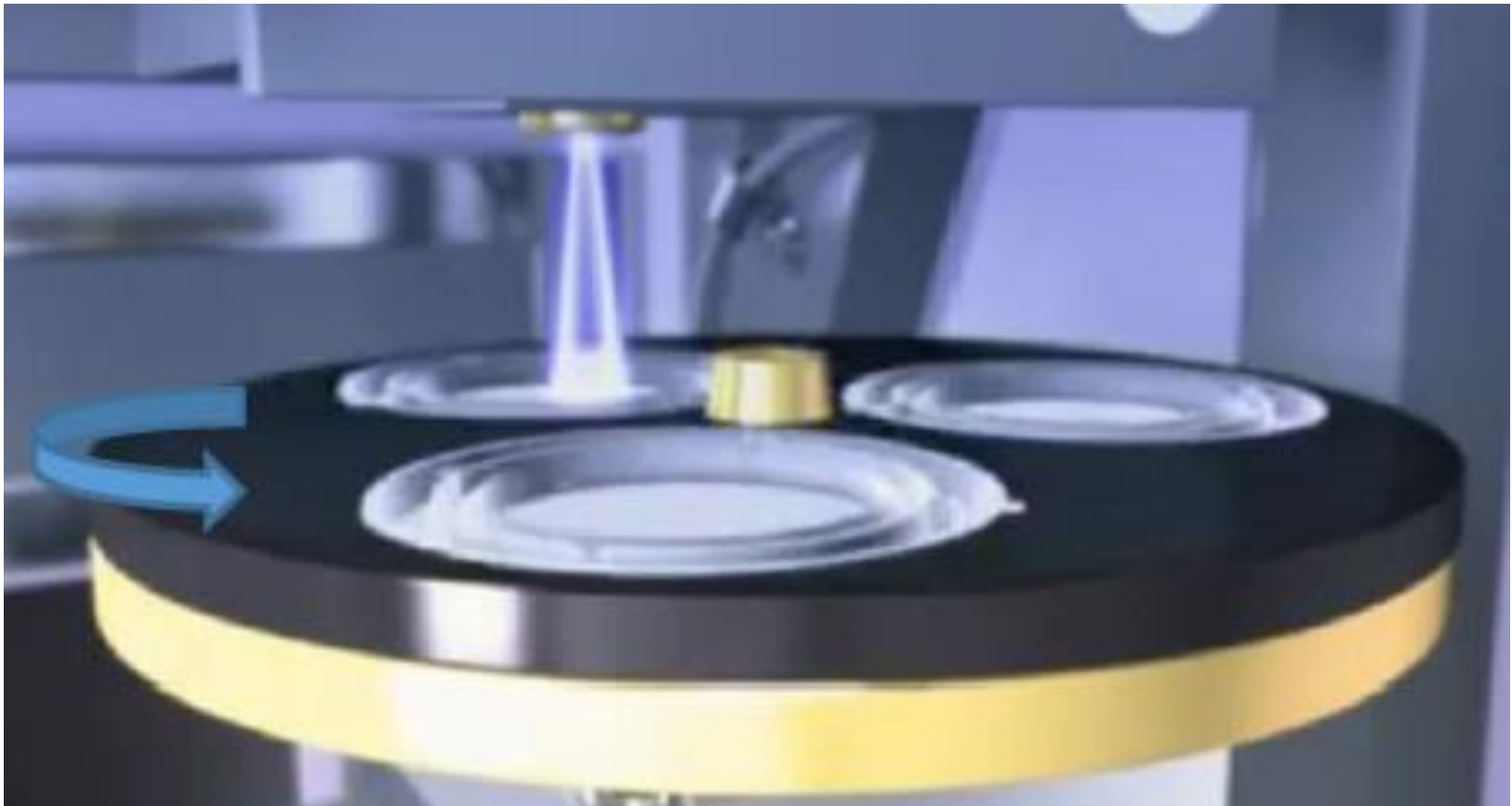


Step 4. Place in Holder and  
Insert into PTS Micro





# Samples in Scanning Mode



# Capillary holder



# PTS Micro – Basic Principle

- Membrane holder spins inside instrument
- Membrane carriage moves past the laser
- Laser excites fluorescent events on the membrane
- Signals emanating from cells are recorded in one of three channels depending on wavelength (color)
  - organisms stained with live cell dye captures in one channel
  - positive control beads captured in separate channel
- Software algorithms discriminate based on size, shape and intensity to exclude background, dead cells, etc



**Solid Phase Cytometry**



# PTS Micro Assay Methods

- **DNA Stain / Quencher**

- Results in as little as 15 min
- Sensitivity measured as viable microorganisms (*VM* / volume )
- Viable but not cultivable
- False positives possible

- **Reductase substrate (CTC)**

- Enrichment 4- 12 hours
- Excellent correlation to cfu
- first commercial application

- **Esterase substrate**


- Results in as little as 30 minutes
- Sensitivity measured as viable microorganisms (*VM* / volume )
- Enrichment not needed
- Couple with spore germination - 2 hour

- **Species Specificity (Future)**

- culture
- fluorescent label - Ab
- Raman spectroscopy (requires second laser module)

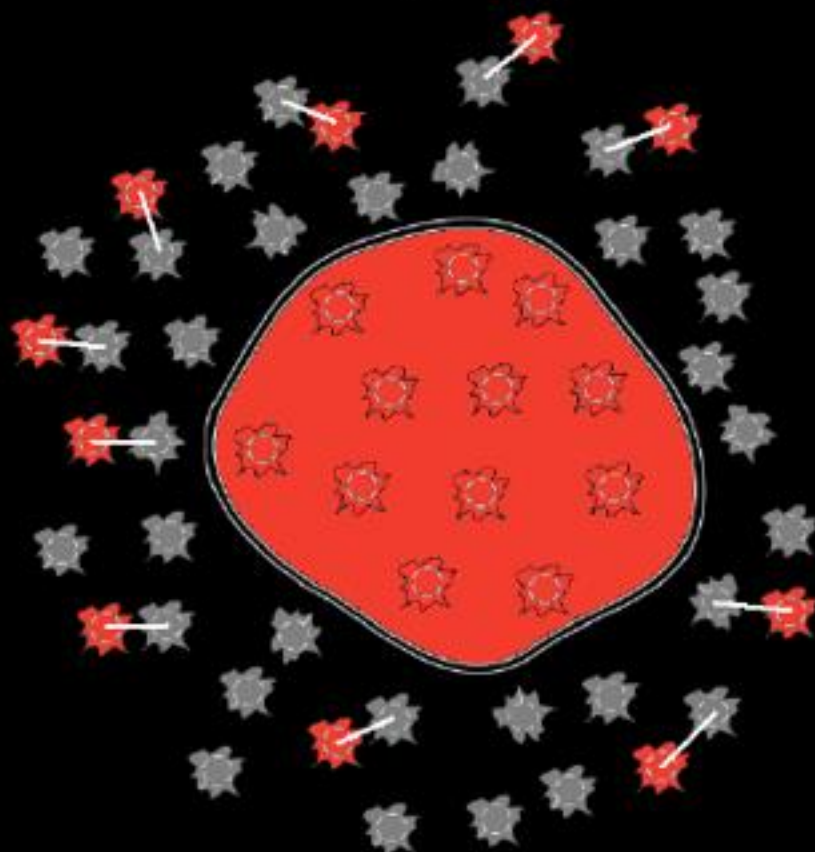
# Live vs. Dead Cell Differentiation

 *Fluorescent dye*

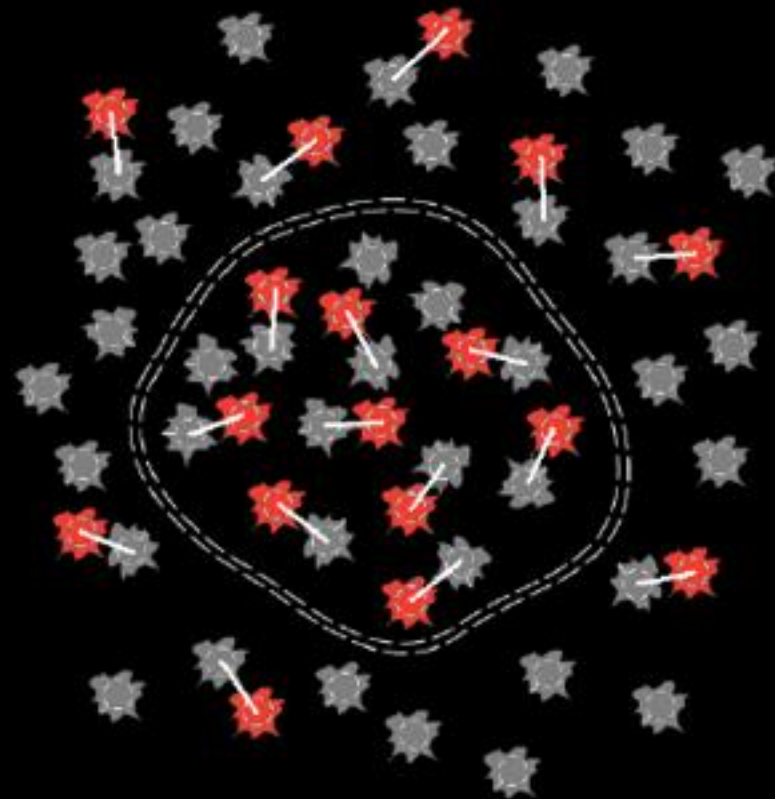
 *Membrane impermeable quencher*

 *Non-Fluorescent Dye-Quencher Complex*

## Live Cell

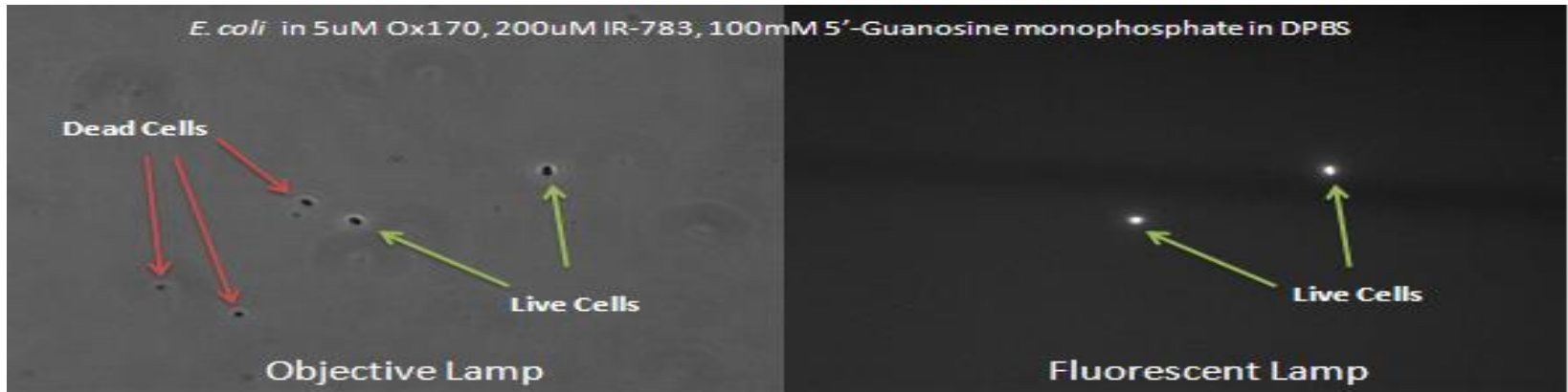


## Dead Cell



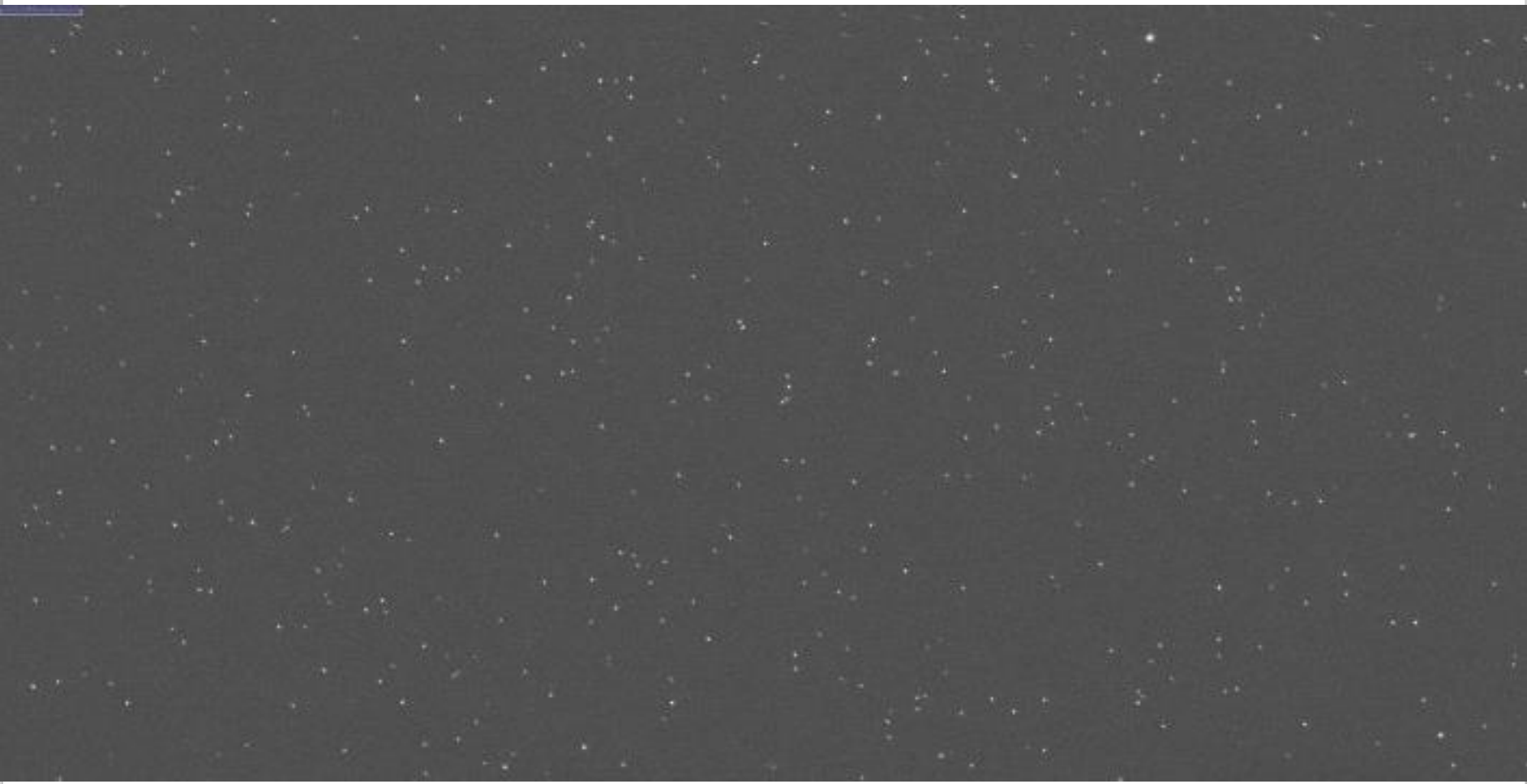
# Live Cell Staining Mechanism

PTS Micro uses a fluorescent live cell stain and a dead cell quencher combination to accurately detect viable microorganisms

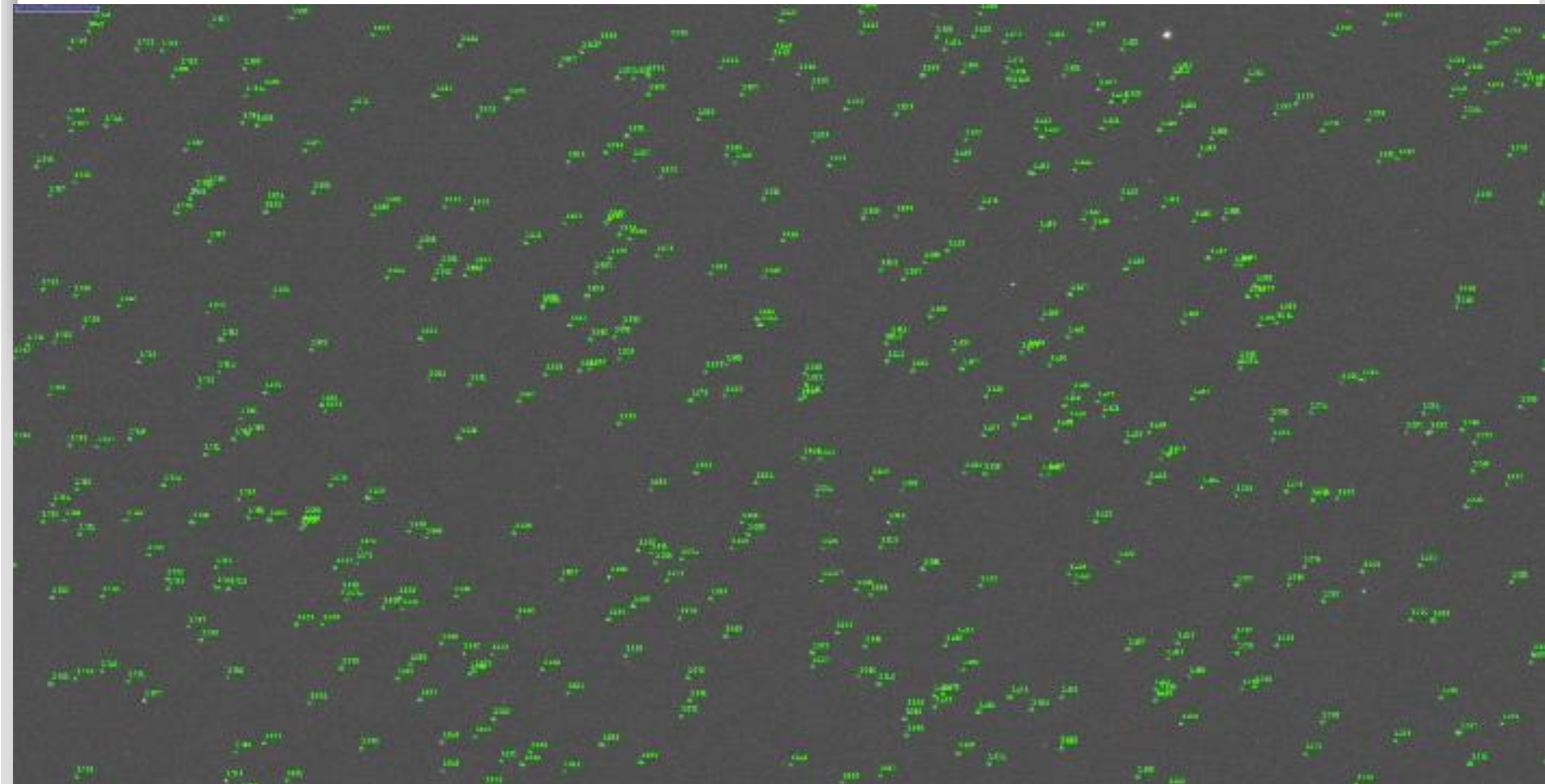




# S. aureus cells - raw data



# S. aureus - software detection



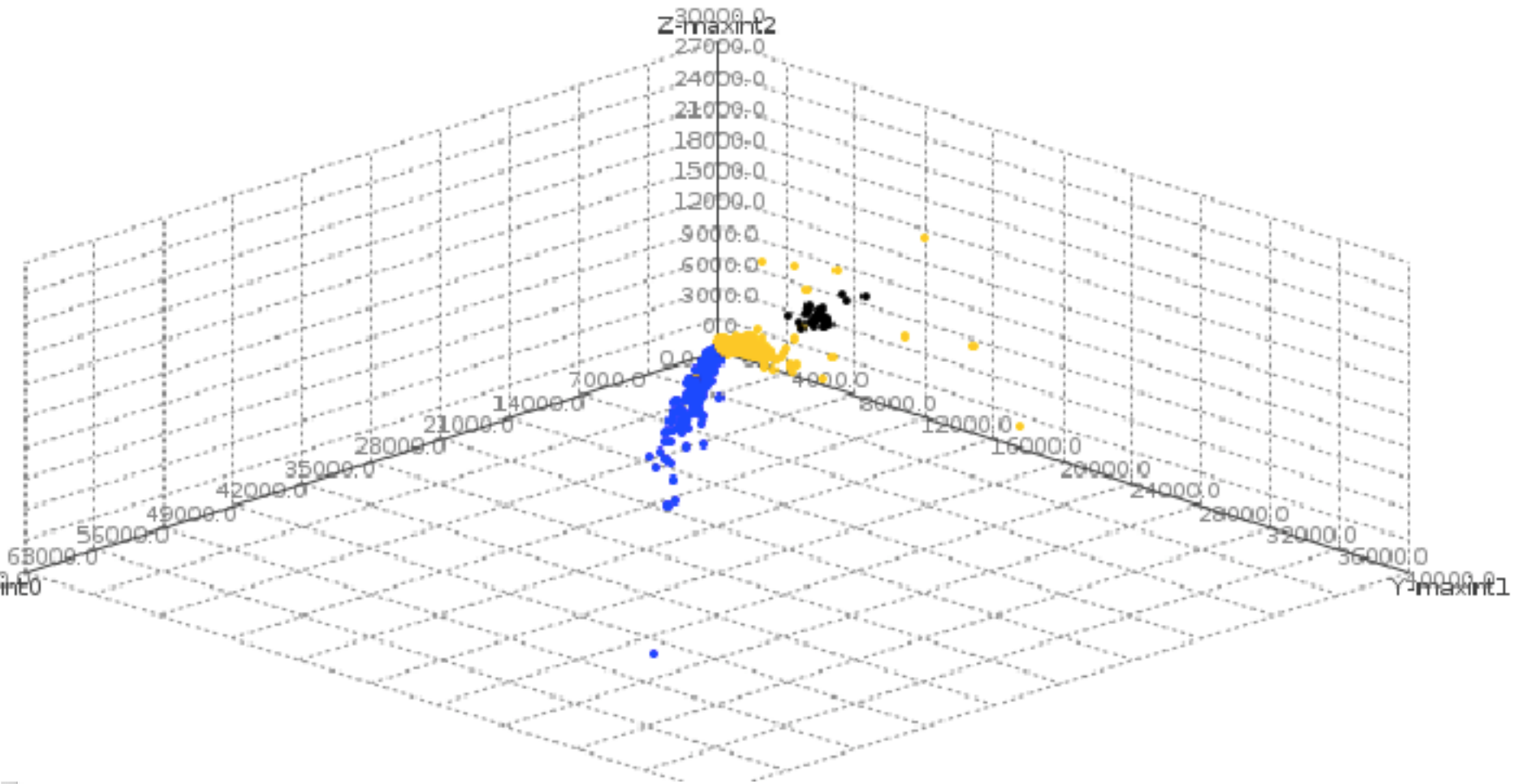
## Amplitude

## Position

$\lambda = 200 \text{ nm}$	400 nm	600 nm	$r \text{ (}\mu\text{m)}$	$\theta \text{ (radians)}$
7830.0	7867.4	7396.2	15210.9	5.1
9216.6	7349.1	9655.0	16757.0	0.4
8839.9	7431.7	8996.7	15598.0	5.5
9201.7	7679.5	9448.6	13723.9	0.3
<b>6522.7</b>	<b>8661.5</b>	<b>10470.6</b>	<b>13719.1</b>	<b>3.3</b>
7569.1	8114.8	7569.4	18119.9	5.5
8507.2	7852.9	8990.1	22616.8	4.3
8173.7	8768.9	8512.3	19772.0	2.6
8369.1	7700.4	5459.9	21685.6	2.1
7967.2	9167.9	7470.4	21397.8	0.2
7915.5	8013.8	7126.7	17034.7	5.2
7739.7	7616.3	8835.9	17075.8	2.4
9589.9	4932.7	7802.4	16925.0	5.8
9662.3	7821.2	9252.9	15840.1	1.4
7589.3	8275.0	8819.4	14236.2	1.8
8304.0	7530.3	6442.6	16005.0	2.4
8640.9	7407.2	6903.2	22526.7	5.7
7430.6	7683.5	7601.5	20098.3	1.5
8152.8	7862.6	6768.5	14238.8	0.1

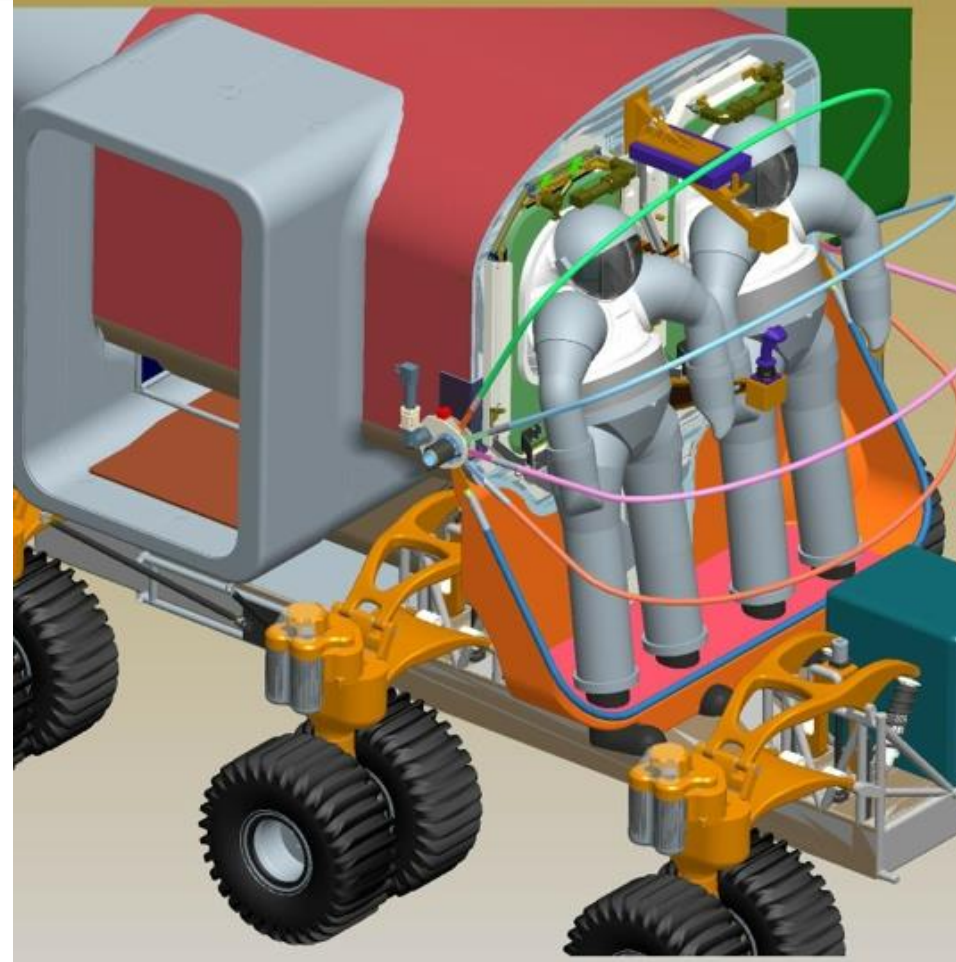


# Plot spectral profile



# checklist

- What planetary protection (PP) related research activities or technical developments do you feel are critical for inclusion in your study area? **Sampling**
- What work/research is already underway? **Hardware / Software**
- Is special information or technology needed to plan for nominal vs. non-nominal situations? **Engineering Integ.**
- Are existing human mission mitigation options and approaches adaptable for PP needs on the martian surface? **Yes**
- Are there any significant stumbling blocks ahead that are evident? (**Including coordination across PP, science exploration, engineering, operation and medical communities.**)
- In your opinion, what still needs to be accomplished? **Consensus**



## Path Forward

- Core technology for rapid viable cell detection maturing
  - Species specificity by fluorescent labeled Ab
  - Species specificity by Raman
- Spacecraft Assembly applications
- Adaptation of Hardware for flight
- Sample Acquisition tools needed
- Potential multi-assay platform